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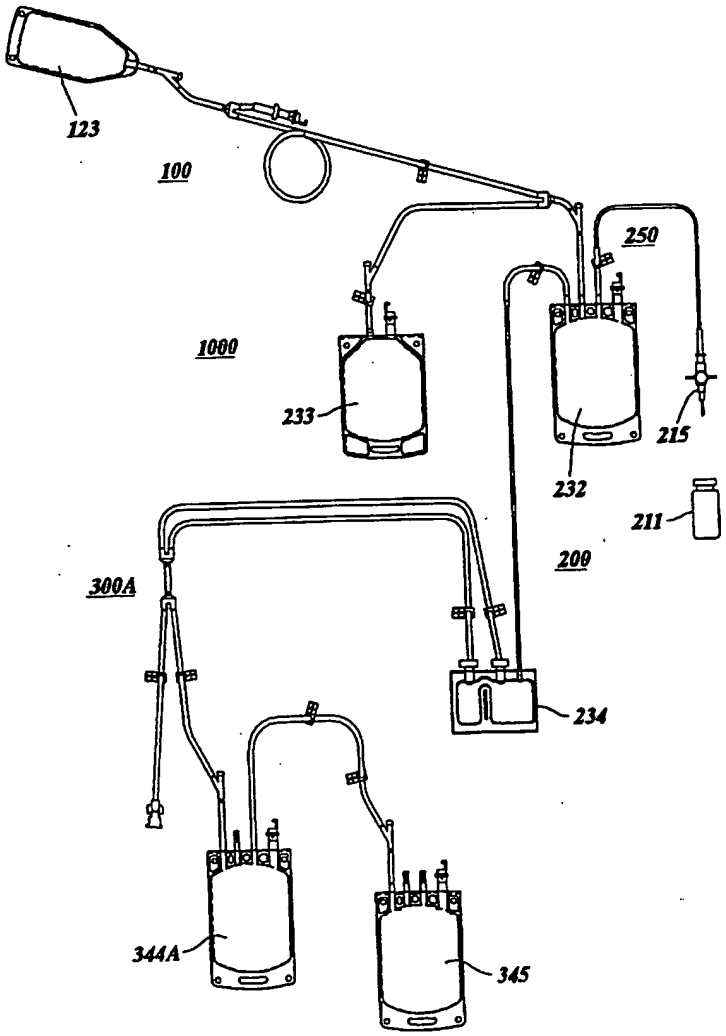
(54) **SYSTEME ET METHODES DE MANIPULATION DE FLUIDES**
(54) **SYSTEM AND METHODS FOR HANDLING FLUIDS**

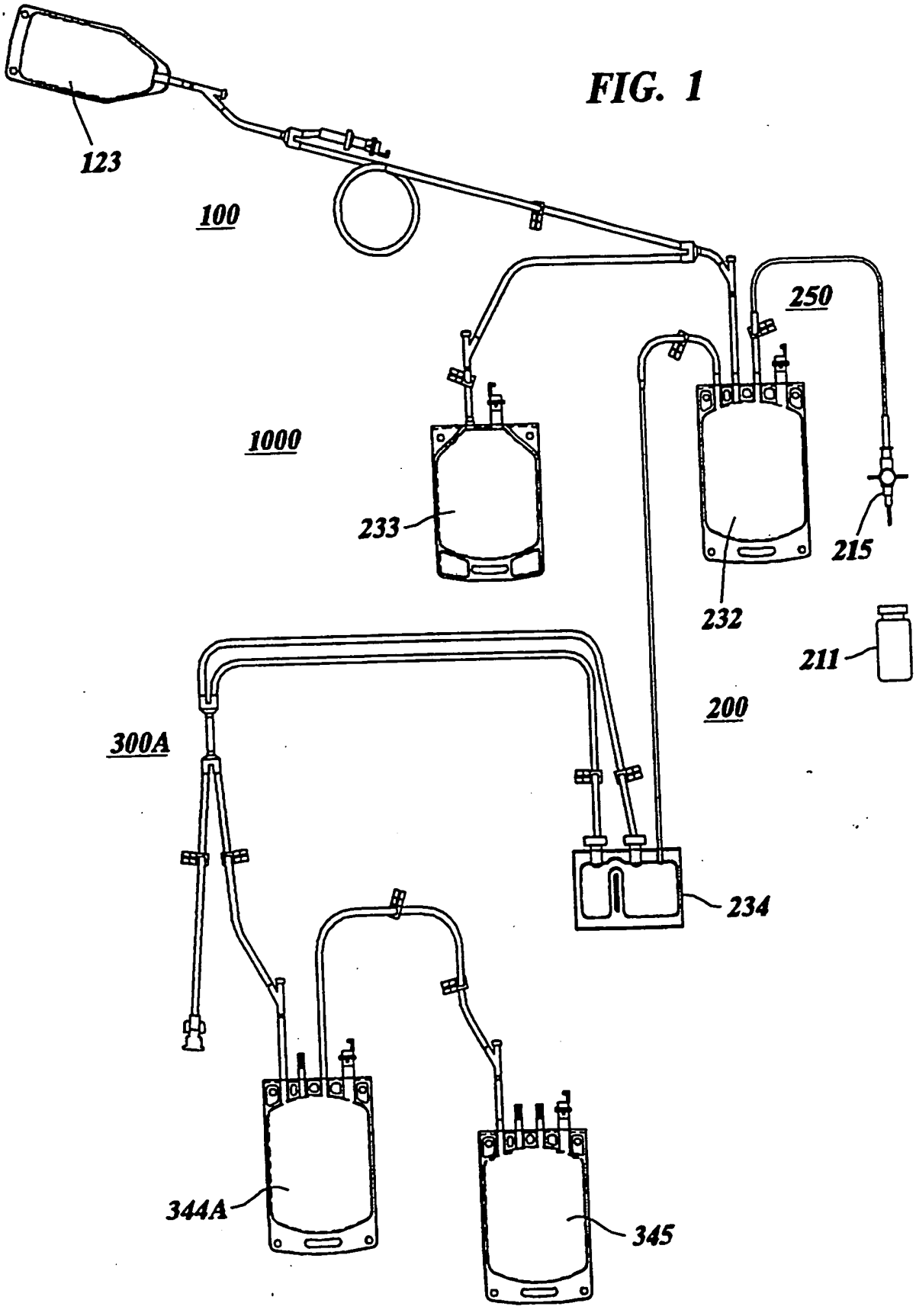


(57) Systems and methods for transferring a fluid (e.g., to process stem cells) in a controlled manner are disclosed.

ABSTRACT

Systems and methods for transferring a fluid (e.g., to process stem cells) in a controlled manner are disclosed.

FIG. 1



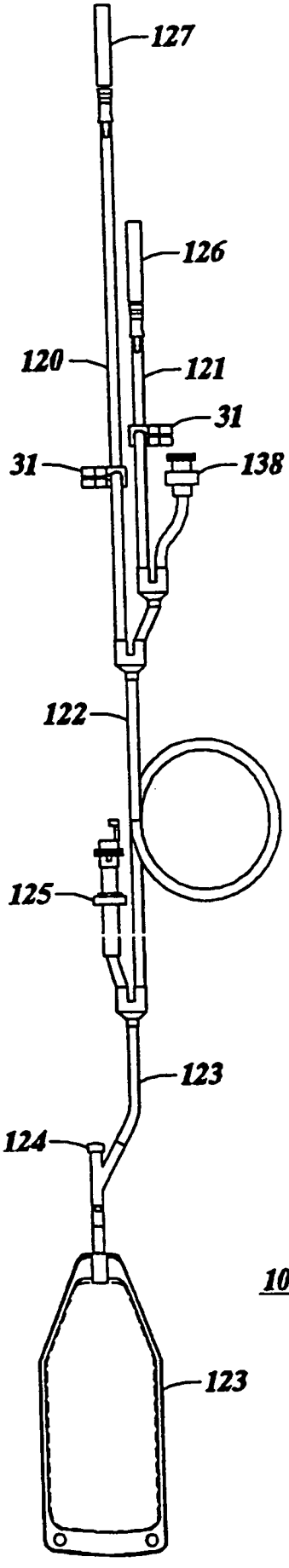
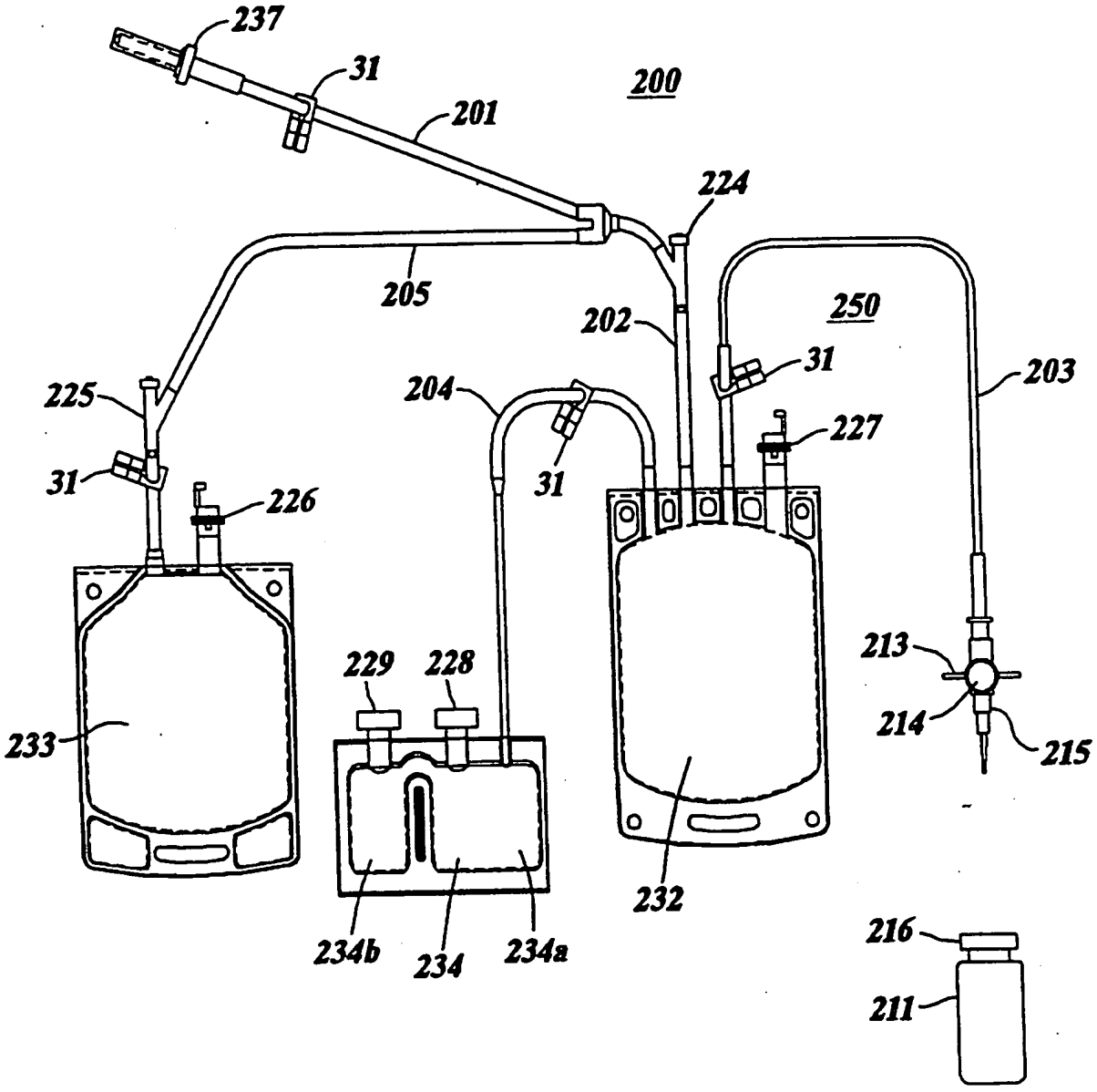


FIG. 2


237
31
200
201
224
205
250
202
31
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225
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213
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232
216
234b
234
234a
211

FIG. 3

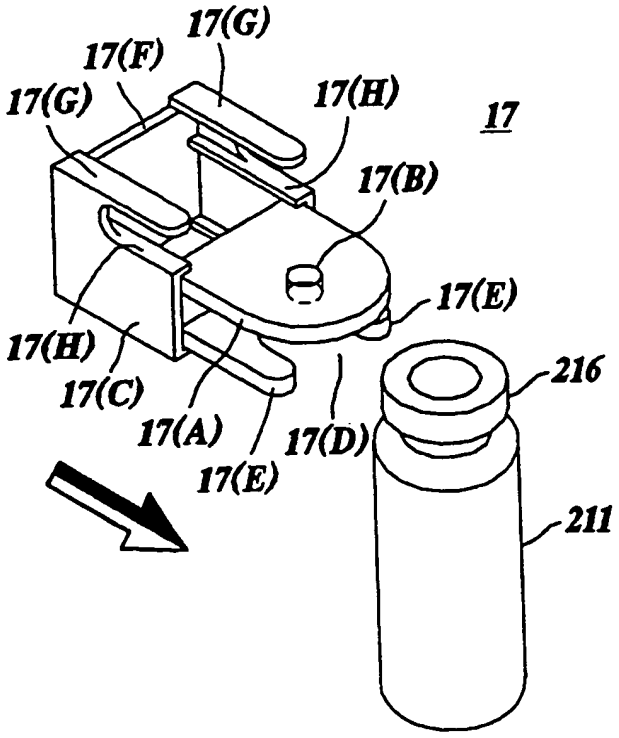


FIG. 4A

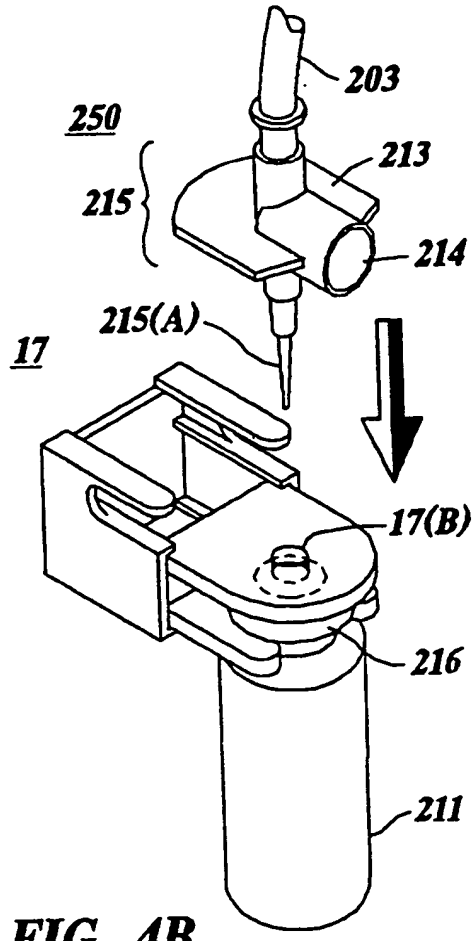


FIG. 4B

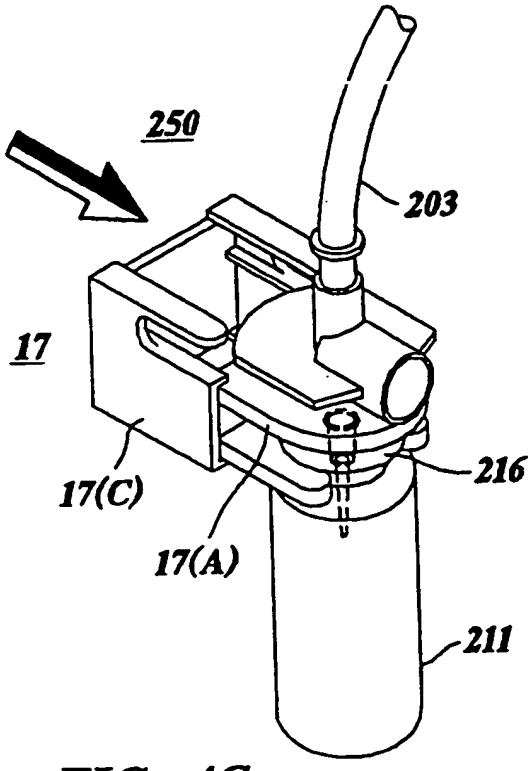


FIG. 4C

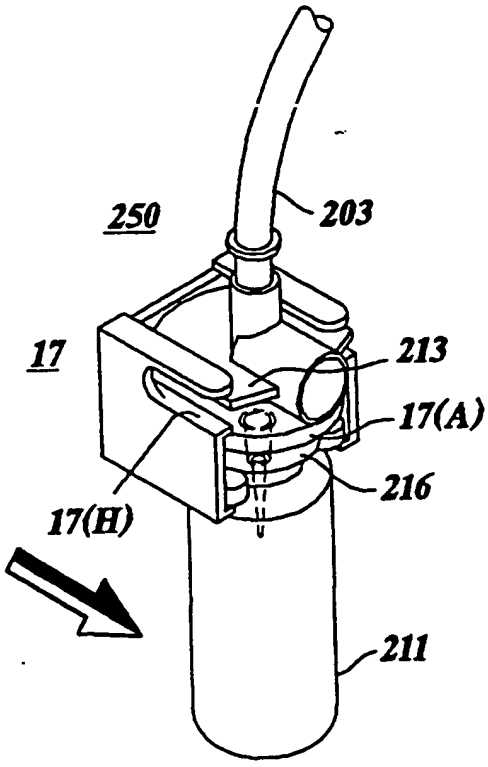


FIG. 4D

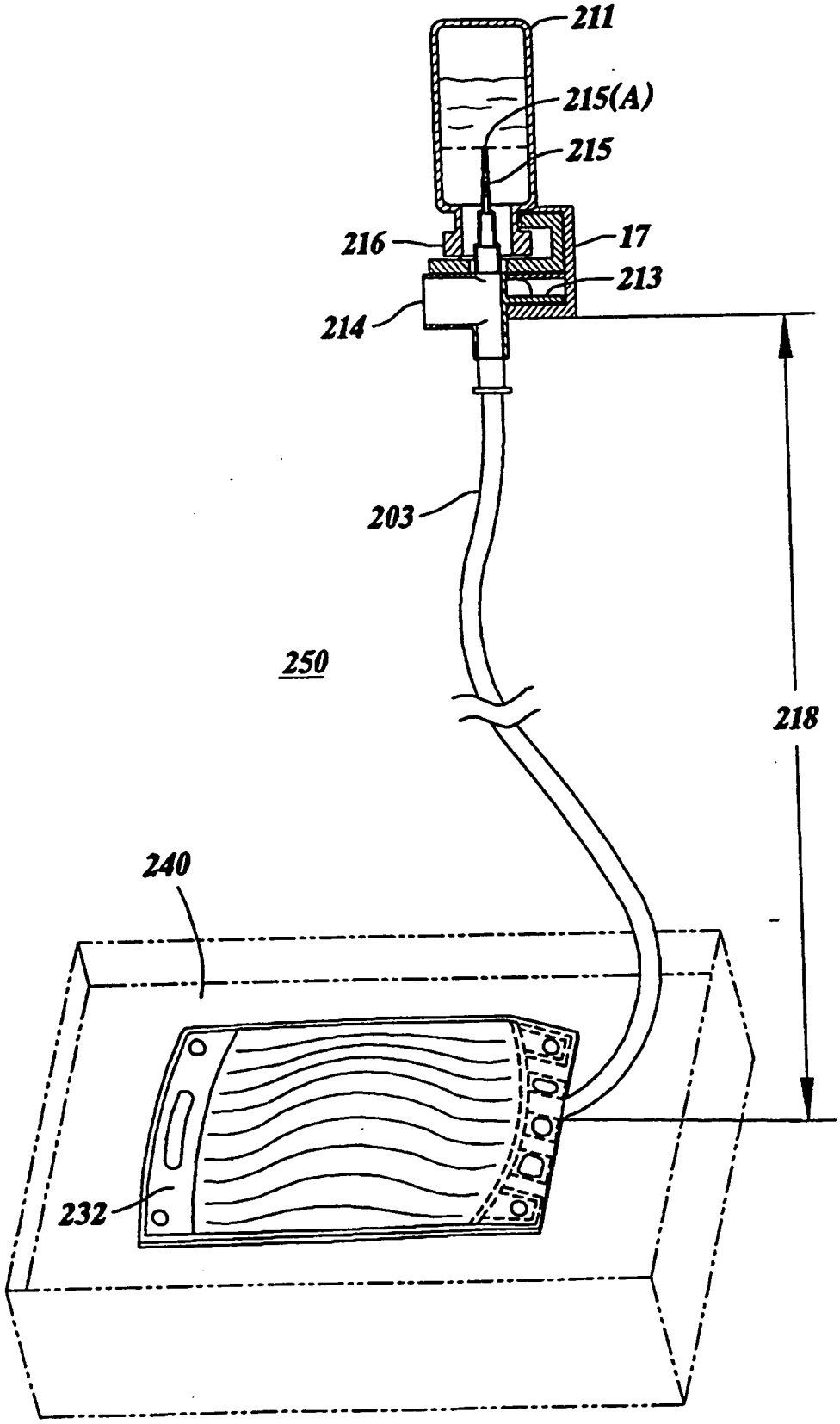
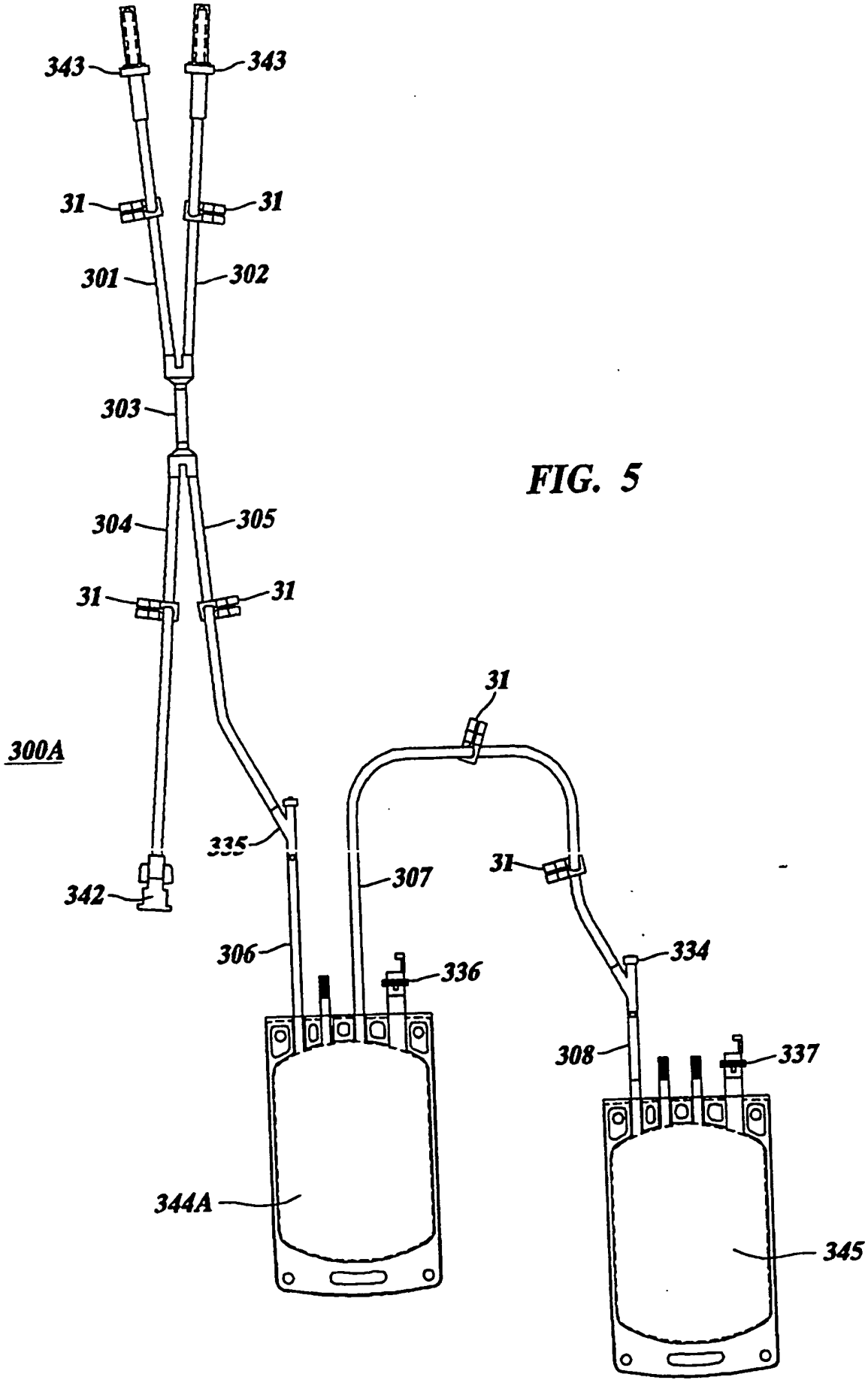


FIG. 4E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300A
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344A
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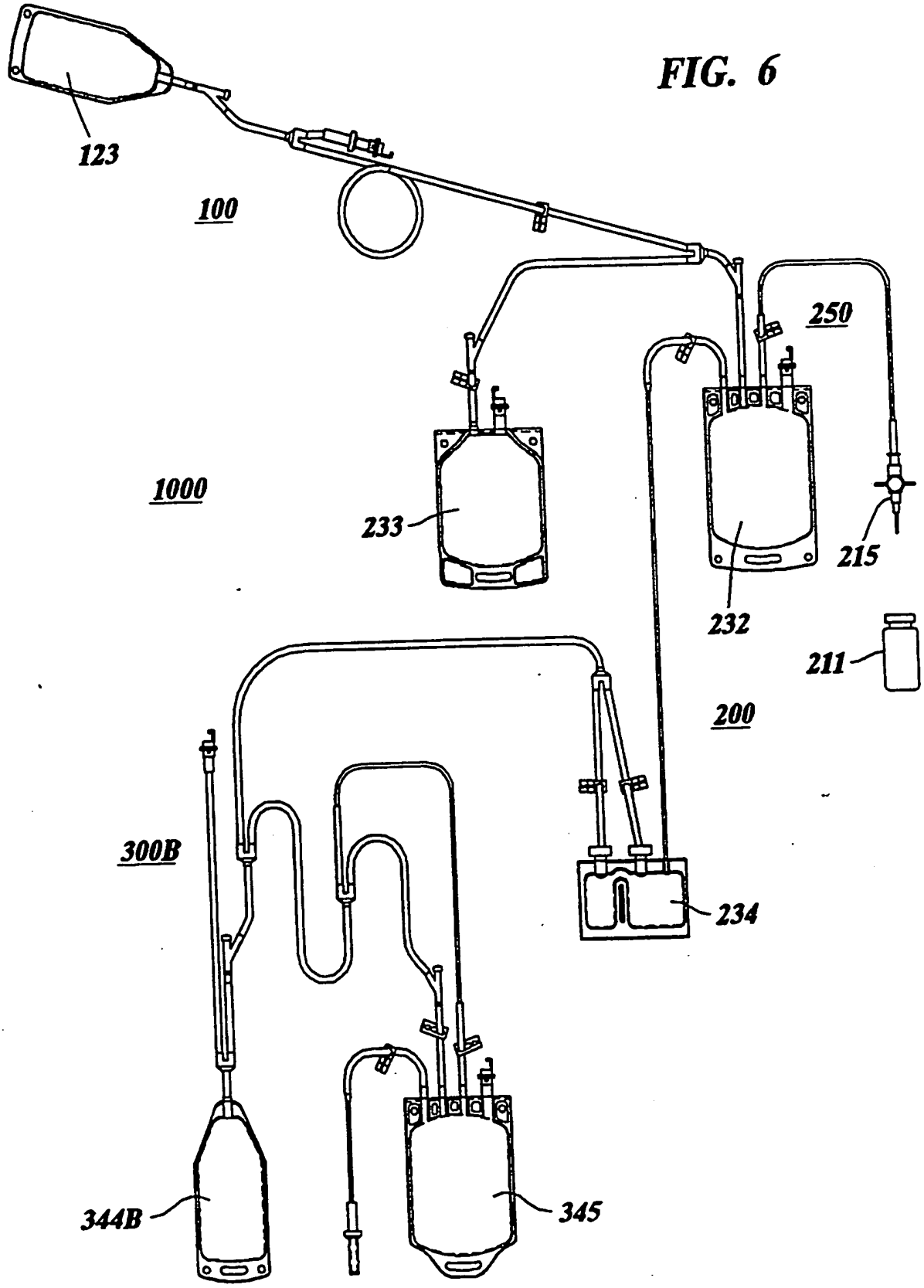
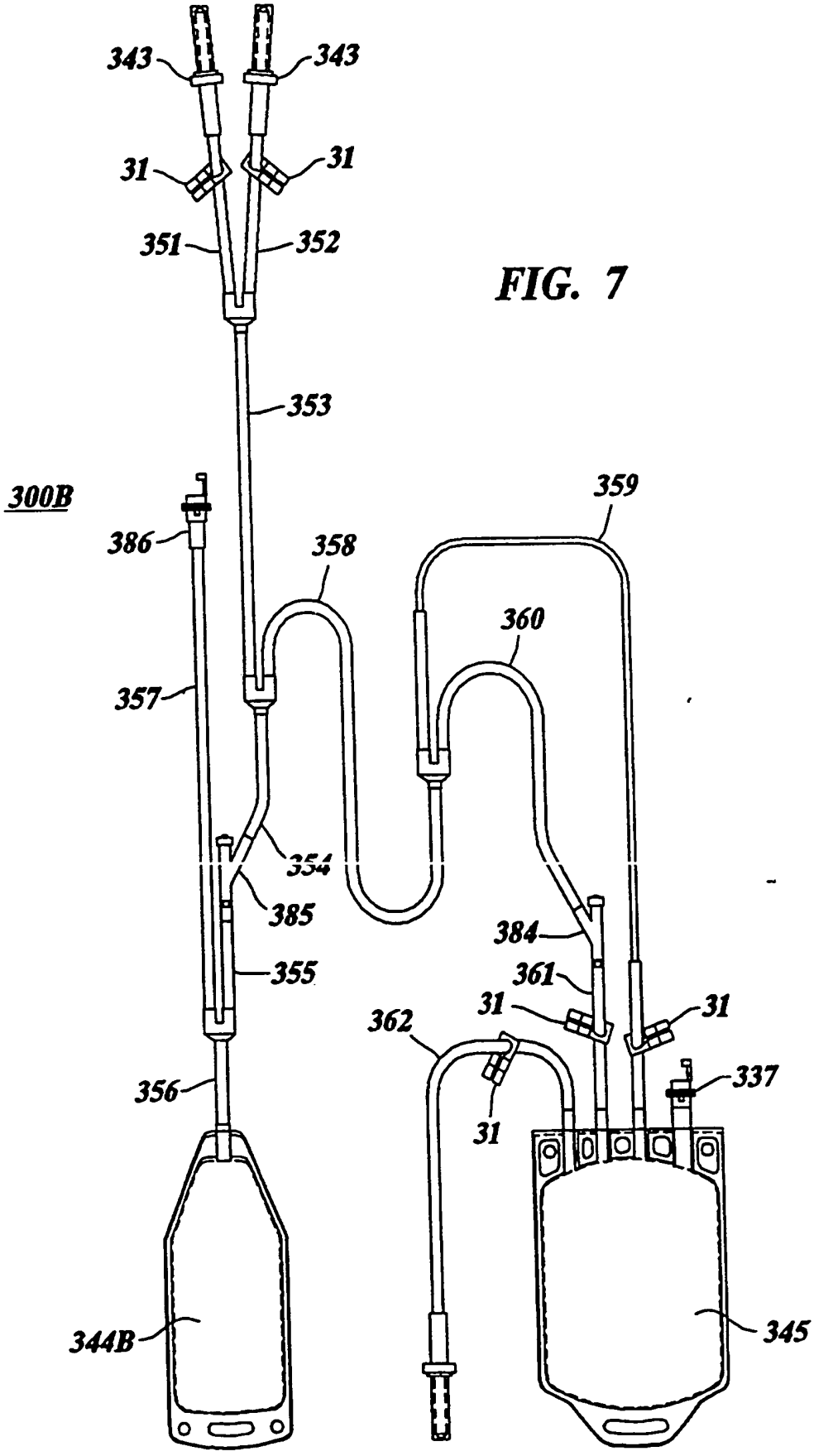
FIG. 5

FIG. 6

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343
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31
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300B
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344B
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FIG. 7

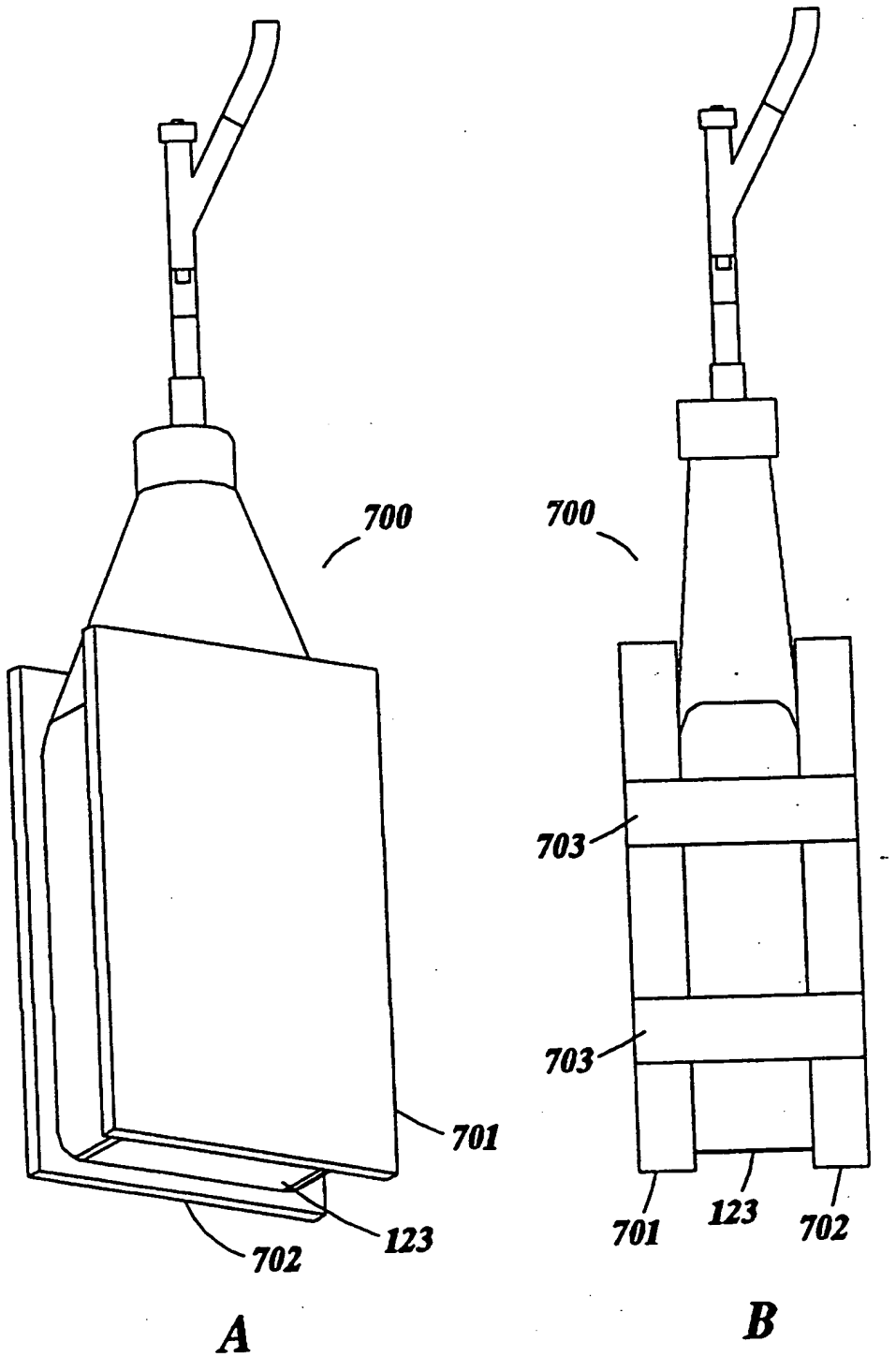

FIG. 8

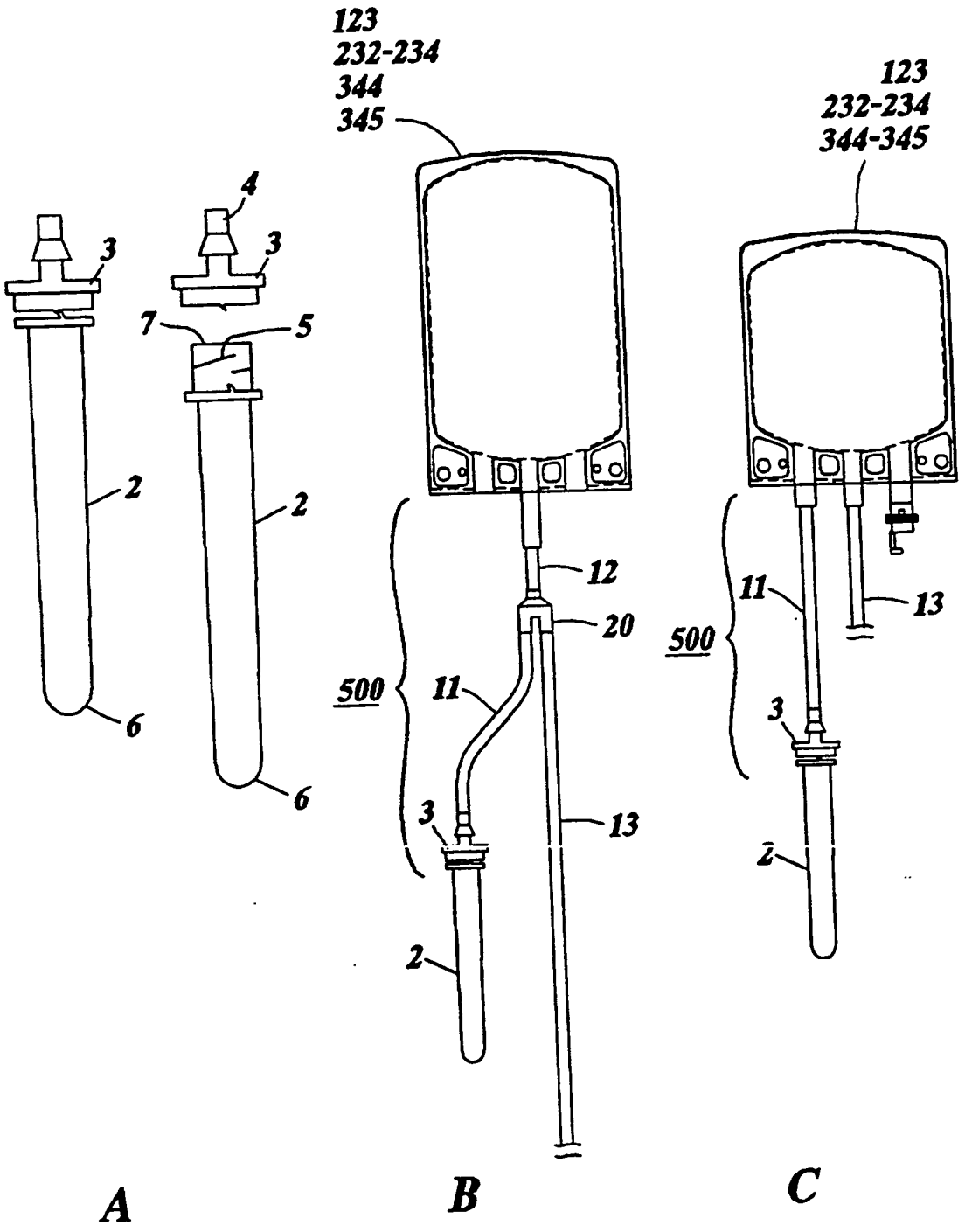
123
232-234
344
345
4
3
3
7
5
2
2
6
6
12
20
500
11
3
13
2
123
232-234
344-345
11
13
500
3
2
A
B
C

FIG. 9

SYSTEM AND METHOD FOR HANDLING FLUIDS

This application claims the priority of U.S. provisional patent application 60/072,468, filed January 26, 1998, which is incorporated by reference.

Technical Field

The present invention relates to systems and methods for handling fluids such as biological fluids, particularly with respect to preserving blood components, such as stem cells.

Background of the Invention

Blood from a newborn's umbilical cord and placenta (hereinafter referred to as "cord blood") includes hematopoietic stem cells. Stem cells (also known as progenitor cells) are capable of forming different types of mature, terminally differentiated blood cells. Due to their undifferentiated nature, stem cells have a variety of therapeutic uses. For example, transfused stem cells can replace blood cells in the bone marrow which may have been destroyed by disease or medical treatment (e.g., radiation).

It would be desirable to create a bank for preserving and storing cord blood. An international bank of cord blood would permit researchers and patients needing stem cells to have quick access to a wide variety of cord blood samples. More importantly, such a bank would permit easy access to a large base of potential matches. However, the creation of an international bank of cord blood requires the collection and storage of vast amounts of cord blood. The collected cord blood would have to be preserved and stored quickly and efficiently.

In conventional processes for collecting, preserving and storing cord blood, placental blood is collected from an umbilical cord and typically processed as follows. The cord blood is collected into a conventional blood bag containing an anti-coagulant, and a sedimentation agent such as hydroxyethyl starch is added to the bag. The bag is then centrifuged to form a white cell-rich supernatant fraction and a red cell-rich sediment fraction, wherein the starch improves the efficiency of red cell sedimentation

and/or aggregation. The white cell-rich supernatant fraction, which comprises white cells (including the stem cells) and plasma, is transferred to another blood bag, and is further centrifuged to form an enriched white cell sediment fraction (also containing some plasma) and a plasma-rich supernatant fraction. The supernatant plasma fraction is removed from the bag, leaving an enriched white cell sediment fraction including the stem cells. This enriched white cell fraction is then combined with a freshly prepared cryoprotectant solution.

The cryoprotectant solution, that is added to the bag containing the enriched white cell fraction, is formed by withdrawing dimethylsulfoxide (DMSO), dextran, and possibly other components, from separate, sterile treatment solution containers to form a mixture. The mixture is subsequently drawn into a syringe, and added to the enriched white cell fraction (through an injection port or site) to the bag.

A stem cell freezing bag is then spike-connected to the enriched white cell bag, and the fluid containing the white cells and cryoprotectant mixture is passed to the stem cell freezing bag. The bag and its contents are then frozen. The stem cells are subsequently thawed and washed (to remove the cryoprotectant) for subsequent uses. For example, the stem cells can be analyzed and/or transfused.

Conventional systems and processes for cryoprotecting cord blood products suffer from a number of disadvantages. For example, cord blood products can be contaminated by microorganisms (particularly bacteria) entering the system during the cryoprotection process, since the formulation of the cryoprotectant mixture and the transfer of the mixture to the enriched white cells is carried out in a compromised or "open" system. The process includes inserting syringes into various injection ports or into sterile containers, each having a different mixture component therein, withdrawing a volume of the component, and adding the components to a mixing container to form the cryoprotectant mixture. The cryoprotectant mixture is then withdrawn from the mixing container and is added to the white cell bag via a syringe. The use of spikes or syringes creates punctures or openings in the various containers and/or injection ports, and these openings expose the contents of the containers (e.g., the cryoprotectant mixture and the stem cells) to potential bacterial contamination from the outside

environment.

Additionally, the various containers in the cord blood handling system are typically connected via spike connectors, which also create punctures or openings that expose the container contents to potential bacterial contamination.

There are other drawbacks to the conventional systems and methods. For example, the processes for formulating the cryoprotectant mixture, and for adding the solution to the white cell bag, are each time and labor intensive, and each aspect relies on the skill of individual operators carrying out these procedures. Additionally, the syringes and pumps used to prepare and add the mixture to the white cell bag may not be calibrated with great accuracy. Since each cryoprotectant solution mixture is prepared and added to the white cell bag individually, the concentration of ingredients in the cryoprotectant mixture, as well as the rate of addition and volume of mixture added to the bag, can vary from operator to operator, from day to day, and from laboratory to laboratory. As a result, it is difficult, if not impossible, to standardize the overall process and system. This variability can adversely affect the quality and/or the yield of the stem cells, and make it difficult to prepare the cells in a uniform, repeatable manner.

Moreover, typical cord blood handling systems, e.g., including conventional blood bags, can be bulky, incompatible and/or inefficient for use in handling stem cells. For example, the volume of cord blood collected is substantially less than the volume of conventional blood bags, making it difficult to centrifuge the bag and/or express the contents of the bag efficiently.

The present invention provides for ameliorating at least some of the disadvantages of the prior art. These and other advantages of the present invention will be apparent from the description as set forth below.

Summary of the Invention

The present invention provides a treatment fluid metering system dimensioned for controllably transferring a treatment solution to a desired location, such as a container, wherein the treatment solution contacts a fluid and/or a substance to be

treated. In a preferred embodiment, the system provides for transferring a controlled predetermined volume of a treatment solution such as a cryoprotectant mixture at a controlled predetermined rate to a container having stem cells disposed therein.

The present invention also relates to systems and methods for handling a fluid, e.g., collecting, processing and freezing at least one desired component of the fluid, and provides for reducing the potential for contamination of the desired component(s) of the fluid during handling. For example, in one embodiment wherein the desired component comprises stem cells, the invention provides for combining the cells with a preformulated sterile treatment solution, e.g., a storage solution such as a cryoprotectant mixture.

In a preferred embodiment, the present invention provides for combining stem cells with a preformulated sterile cryoprotectant mixture, wherein the mixture is controllably transferred to a container having the cells disposed therein. In one embodiment, the system provides for transferring a cryoprotectant mixture to a container having stem cells therein while maintaining a closed system.

Brief Description of the Drawings

Figure 1 illustrates a schematic view of an embodiment of a fluid handling system according to the invention, including a collection set, a processing set, and a post thaw set.

Figure 2 illustrates an embodiment of the collection set shown in Figure 1.

Figure 3 illustrates an embodiment of the processing set shown in Figure 1, showing a treatment solution metering system for connection to a container of treatment solution.

Figures 4 A-E illustrate, in perspective views, providing fluid communication between the treatment solution metering system and the container of treatment solution shown in Figure 3.

Figure 5 illustrates an embodiment of the post thaw set shown in Figure 1.

Figure 6 illustrates a schematic view of another embodiment of a fluid handling system according to the invention, including a collection set, a processing set, and a

post thaw set.

Figure 7 illustrates an embodiment of the post thaw set shown in Figure 6.

Figures 8 A-B illustrate an embodiment of a device for centrifuging the collection set.

Figures 9 A-C illustrate embodiments of a sampling arrangement for taking a sample in accordance with the invention.

Specific Description of the Invention

In an embodiment of the invention, a fluid metering system is provided comprising a connector suitable for providing fluid communication with a first container, the first container comprising a treatment solution container having a volume of treatment solution disposed therein, and a conduit in fluid communication with the connector, wherein the system is dimensioned for transferring from the first container into a second container downstream of the conduit a controlled predetermined volume of the treatment solution that is less than the volume of the treatment solution in the first container.

In another embodiment, a fluid metering system comprises a connector suitable for providing fluid communication with a first container, the first container comprising a treatment solution container and having a defined internal volume, and a conduit in fluid communication with the connector, wherein the system is dimensioned for transferring from the first container into a second container downstream of the conduit a controlled predetermined volume of the treatment solution that is less than the internal volume of the treatment solution container.

An embodiment of a fluid metering system according to the invention comprises a connector suitable for providing fluid communication with a first container, the first container comprising a treatment solution container having a volume of treatment solution disposed therein, and a conduit in fluid communication with the connector, wherein the system is dimensioned for transferring from the first container into a second container downstream of the conduit a controlled predetermined volume of the treatment solution at a controlled predetermined rate, the controlled predetermined

volume being less than the volume of the treatment solution in the first container.

Other embodiments of the fluid metering system are dimensioned for transferring a controlled predetermined volume of treatment solution wherein the controlled predetermined volume substantially comprises the volume of treatment solution in the first container.

In accordance with another embodiment, a fluid metering system comprises a connector suitable for providing fluid communication with a first container, the first container comprising a treatment solution container having a volume of treatment solution disposed therein, and a conduit in fluid communication with the connector, wherein the system is dimensioned for transferring from the first container into a second container downstream of the conduit the treatment solution at a controlled predetermined rate.

A biological fluid treatment system according to an embodiment of the invention comprises a sealed terminally sterilized container, and a treatment fluid mixture including a cryoprotectant, wherein the sealed terminally sterilized container has the fluid mixture disposed therein.

An embodiment of a method according to the invention provides a method for transferring a treatment solution comprising passing a controlled predetermined volume of a treatment solution (e.g., from a first container) through a fluid metering system. The controlled predetermined volume can be less than, or substantially equal to, the volume of treatment solution in the first container

In another embodiment, a method for transferring a treatment solution comprising passing a treatment solution through a fluid metering system at a controlled predetermined rate. In a preferred embodiment, the method provides for passing a controlled predetermined volume of the treatment solution through the metering system at a controlled predetermined rate.

In accordance with an embodiment of the invention, a set for processing a white blood cell-containing fluid comprises a first container plasticized with triethylhexyltrimellitate, and a second container suitable for freezing white blood cells, wherein said second container is in fluid communication with the first container.

The following definitions are used in accordance with the invention.

(A) Fluid. Fluid includes any liquid, or gas, or mixtures thereof. In a preferred embodiment, the fluid is a biological fluid, as defined below. Other suitable fluids include various suspensions or solutions. In an illustrative embodiment, the fluid comprises a tissue culture fluid, which may include, for example, one or more proteins such as hormones, enzymes, cell expression products, and/or one or more cell types.

(B) Biological Fluid. A biological fluid includes any treated or untreated fluid associated with living organisms, particularly blood, including whole blood, blood from the placenta, blood from the umbilical vessels, cord blood (i.e., blood from the placenta and the umbilical cord), warm or cold blood, and stored or fresh blood; treated blood, such as blood diluted with at least one physiological and/or storage solution, including but not limited to saline, nutrient, anticoagulant and/or cryoprotective solutions; blood components, such as platelets, platelet concentrate (PC), platelet-rich plasma (PRP), plasma, components obtained from plasma, packed red cells (PRC), buffy coat (BC); blood products or blood components derived from blood (including peripheral blood and cord blood) and/or bone marrow; blood components such as white cells, platelets and/or red blood cells separated from plasma and resuspended in a physiological fluid or a cryoprotective fluid. The biological fluid may have been treated to remove some of the components before being processed according to the invention. As used herein, blood product or biological fluid refers to the components described above, and to similar blood products or biological fluids obtained by other means and with similar properties. Preferably, the biological fluid includes cells, especially stem cells.

(C) Unit. Typically, a unit of cord blood is the quantity collected or drawn from a single placenta via the umbilical cord. A unit can also refer to the quantity of biological fluid drawn from a donor or derived from one unit of whole blood. The volume of a unit typically varies, differing from collection to collection. Multiple units of some blood components, particularly platelets and buffy coat, may be pooled or combined, typically by combining four or more units.

Each of the components of the invention will now be described in more detail

below, wherein like components have like reference numbers. Figures 1 and 6 illustrate embodiments of system 1000 for handling biological fluid, including a collection set 100, a processing set 200 including a treatment solution metering system 250, and a post thaw set 300A (Figure 1) or 300B (Figure 6). Embodiments of the collection set 100, the processing set 200 including the treatment solution metering system 250, and post thaw set 300A and 300B are shown in more detail in Figures 2-5 and 7, respectively.

In the following general description, the embodiments of the system 1000 as illustrated in Figures 1 and 6 can be utilized similarly, unless noted otherwise, e.g., with respect to the embodiments of post thaw set 300A and 300B.

Using the illustrated embodiments of Figures 1 and 2 for reference, biological fluid, e.g., cord blood, is collected in collection container 123 in the collection set 100 (Figure 2), and processed to form a red cell-rich sediment fraction and a white cell-rich supernatant fraction. Collection set 100 (having the cord blood therein) is connected to the rest of the system 1000 (Figure 1).

Turning now to Figures 1 and 3, the white cell-rich supernatant fraction is passed from collection set 100 into the receiving container 232 in processing set 200 including treatment solution metering system 250. The white cell-rich fluid in receiving container 232 is further processed to provide a white cell-enriched, plasma volume-reduced fluid comprising stem cells, and the majority of the plasma is subsequently passed into plasma container 233. The white cell-enriched plasma volume-reduced fluid remains in receiving container 232, and a treatment solution (e.g., a preformulated sterile cryoprotectant mixture contained in treatment solution container 211) is added to the white cell-enriched fluid in receiving container 232 through treatment solution metering system 250.

The treatment solution metering system 250 provides for metering the flow of treatment solution from the container 211 into the receiving container 232. Figures 4 A-E illustrate placing treatment solution container 211 in fluid communication with treatment solution metering system 250 via connector 215.

Turning back again to Figure 3, the white cell-enriched fluid, now mixed with,

and suspended in, treatment solution, is passed into storage container 234, and the fluid is frozen in the container.

Subsequently, and now using Figures 1 and 5 for reference, the frozen treatment solution/white cell-enriched fluid is subsequently thawed and passed from the freezing bag 234 (from processing set 200) into transplant container 344A in post thaw set 300A where the stem cells are washed (e.g., to deplete the treatment solution from the cells) before further use. Alternatively, using another embodiment shown in Figures 6 and 7 for reference, the thawed fluid is passed from freezing bag 234 into post thaw set 300 B (Figures 6 and 7). Typically, further use of the cells includes analysis, manipulation (e.g., cloning and gene therapy) and/or transfusion.

The components of the fluid handling systems are described in more detail below, initially focussing on the processing set.

PROCESSING SET and TREATMENT SOLUTION METERING SYSTEM

Figures 1 (and 6), 3 and 4 illustrate embodiments of a processing set 200 including a treatment solution metering system 250 as part of a biological fluid handling system 1000.

For convenience, the set and system will be described below for preparing stem cells for storage, and for controllably adding a cryoprotectant mixture to a container having the cells disposed therein. However, the set and metering system can also be utilized in a variety of other applications and protocols, that can involve biological fluid or non-biological fluid. Exemplary applications and protocols include, but are not limited to, preparing therapeutic agents such as drugs and/or nutrients, adding an anticoagulant to a blood collection bag, preparing eggs and/or sperm, and handling other fluids such as tissue culture fluids. Other applications and protocols can include, for example, treating cells and/or preparing cell-free solutions or suspensions.

In these illustrated embodiments, the processing set 200 includes a treatment solution metering system 250, a plurality of conduits 201-205, and a plurality of containers 232-234. For convenience and clarity, the containers will be referred to below as the receiving container (or the white cell container) 232, the plasma container

233, and the freezing bag 234. The illustrated set 200 also includes access ports 224-229 (such as injection ports and/or connector ports), and flow control devices 31. Exemplary flow control devices, that can be adjustable flow devices, include, but are not limited to, clamps (including adjustable clamps such as screw clamps), valves, and the like.

Additionally, as shown in Figures 3, 4B, and 4E, an embodiment of the set 200 also includes a treatment solution container 211, having a treatment solution (e.g., a cryoprotectant mixture) disposed therein.

Figure 4E illustrates an embodiment of the treatment solution metering system 250 in processing set 200 in more detail, wherein the system 250 is disposed at a predetermined head height 218 during use. The illustrated embodiment of the treatment solution metering system 250 comprises connector 215 including a base 213, at least one conduit 203 in fluid communication with the connector 215, and a vent 214. The connector 215 provides fluid communication between the rest of the system 250 and the treatment solution container 211.

In the illustrated embodiment, connector 215 comprises a penetrating connector, such as a spike or needle having a predetermined length, and conduit 203 comprises tubing (e.g., microbore tubing) having a predetermined length and inner diameter. In another embodiment (not shown), the connector comprises a length of tubing (such as microbore tubing) wherein placing the connector in fluid communication with the treatment solution container includes sterile docking, e.g., as disclosed in U.S. Patent No. 4,610,670. Illustratively, a conduit similar to conduit 203 can be "sterile docked" to another conduit that was previously attached to the treatment solution container.

Turning again to Figure 4E, the connector 215 can be attached to, or integrally formed with, the base 213. The base 213 is typically wider than the width of the rest of the connector. In those embodiments wherein connector 215 comprises a penetrating connector having a penetrating end 215(a) (e.g., as shown in Figures 4B and 4E), the system 250 typically also includes a fitting or jig 17, e.g., to provide an interface between the connector 215 and the container 211. If desired, the jig can

engage with the connector 215 (for example, by engaging with base 213) and/or the container 211 (for example, by engaging with shoulder 216).

In those embodiments wherein the system includes a vent 214, e.g., wherein the treatment solution container 211 comprises a substantially non-collapsible container, the vent preferably comprises a filter that allows air or gas (but not bacteria) to pass into the container 211, and resists the passage of liquid therethrough.

If desired, the set can also include at least one additional component such as but not limited to, at least one filter such as a bacterial filter (not shown), for example, interposed between the treatment solution container 211 and receiving container 232 for filtering the treatment solution. Other illustrative additional components include, but are not limited to, at least one of a vent, a container, a conduit, a transfer leg closure, and a sampling arrangement.

The treatment solution metering system 250 shown in Figure 3 and 4E is dimensioned for transferring the treatment solution from container 211 into receiving container 232 (that contains white cells including stem cells disposed therein) in a controlled manner. For example, as will be described in more detail below, the metering system 250 provides for passing into the receiving container a controlled predetermined volume of cryoprotectant mixture at a controlled predetermined rate.

Typically, the stem cells are processed (e.g., concentrated) before transferring the cryoprotectant mixture into the receiving container 232. For example, processing set 200 (shown in Figure 3), that includes metering system 250, is first placed in fluid communication with a collection set 100 (shown in Figures 1 and 2), so that the white cells (including the stem cells) suspended in plasma can be passed from the collection set 100 into the processing set 200 via conduits 201 and 202. If desired, the set 200 can be placed in fluid connection with set 100 via spike 237 (Figure 3). Alternatively, the sets can be connected while maintaining a closed system, e.g., via sterile docking.

Referring now to Figure 3, the white cells suspended in plasma in receiving container 232 are centrifuged to form a supernatant fraction comprising plasma, and a sediment fraction comprising white cells and some plasma, wherein the white cells include the stem cells. The supernatant fraction is passed from receiving container 232

into plasma container 233 via conduits 202 and 205, leaving a white cell-enriched, plasma volume-reduced fluid in the receiving container (or white cell container) 232.

The treatment solution metering system 250 is placed in fluid communication with the treatment solution container 211 via connector 215. In accordance with the invention, the treatment solution metering system 250 is dimensioned for transferring the treatment solution from container 211 into the white cell container 232 (that contains the stem cells therein) in a controlled manner.

For example, in one embodiment, the metering system 250 provides for transferring a controlled predetermined volume of treatment solution from the treatment solution container 211 into the white cell container 232. Typically, the transferred controlled predetermined volume is less than the volume of treatment solution in the container 211. Illustratively, and using the embodiment illustrated in Figure 4E for reference, the flow of cryoprotectant mixture from the container 211 through conduit 203 into white cell container 232 will stop once the level of fluid no longer covers the tip 215(a) of the connector 215, even though fluid remains in the container 211. Thus, while the container still contains fluid, the flow stops predictably, and automatically, after delivering a predetermined volume of fluid, without manually clamping (e.g., constricting the inner diameter of) the conduit 203 to control the volume of fluid delivered.

In one embodiment wherein metering system 250 does not include a penetrating connector, e.g., wherein container 211 (containing a predetermined volume of fluid) can be connected to the system 250 via sterile docking, the flow stops automatically with treatment solution remaining in the container. For example, the container 211 can comprise a non-vented, non-collapsible container wherein a predetermined residual volume will remain in the container. Alternatively, for example, in some of those embodiments wherein container 211 is vented and/or is collapsible, the flow will stop when the container is empty, e.g., wherein essentially no treatment solution remains in the container.

The treatment solution metering system 250 also provides for transferring the treatment solution from the treatment solution container 211 into the receiving

container 232 at a controlled predetermined rate. Illustratively, in an embodiment, the system includes at least one conduit with a selected inner diameter (e.g., to provide resistance) and the set is disposed at a selected head height during use to provide any desired controlled predetermined flow rate. Accordingly, embodiments of the metering system provide a desired controlled predetermined flow rate with and without penetrating connectors, and without manually clamping a conduit to control the rate. Figure 4E illustrates one embodiment of the system arranged at a predetermined head height 218.

As noted earlier, the treatment solution metering system according to the invention can be utilized in a variety of applications and protocols. For example, since the viscosity of a desired treatment solution is known or can be determined, and the flow rate is dependent on the viscosity and resistance, the system can be dimensioned for transferring the desired treatment solution in a controlled manner. In accordance with the invention, an exemplary controlled predetermined flow rate is in the range of about 0.1 ml/min to about 1 ml/min, or more. In some embodiments, e.g., some embodiments wherein the treatment solution comprises a cryoprotectant mixture, the predetermined flow rate is in the range of about 0.2 ml/min to about 0.5 ml/min.

If desired, as shown in Figures 4 A-E for example, a jig 17 can be interposed between the connector 215 and the container 211, e.g., to guide (more preferably, to more precisely define) the connection between the connector 215 and the container 211. The jig 17 shown in these illustrated embodiments includes an extended portion 17(a) having a controlled thickness, and an aperture 17(b). The jig also includes side walls 17(c), a first open area 17(d) between extensions 17(e), and a second open area 17(f) between extensions 17(g) and slots 17(h). Embodiments of the jig can include moveable elements. For example, as shown in Figures 4C and 4D, one section of the jig, that includes 17(a) and 17(e), moves relative to another section, that includes 17(c) and 17 (g).

In an illustrative embodiment, the jig 17 can reproducibly control the depth of needle insertion, and the alignment of the needle with the stopper of the container 211. If desired, the jig can "lock" or "fix" the connector into place, e.g., by engaging with

the connector base 213, and with a shoulder 216 or groove in the container 211. After the fluid has been transferred from the container 211, the jig is typically disengaged from the container and the connector, and the jig can be used with another treatment solution metering system. The jig can be used with a plurality of treatment solution metering systems with accurate, reproducible, and predictable results (e.g., transfer of a controlled predetermined volume of fluid).

Since the treatment solution is added to the white cell container containing the stem cells in a controlled predetermined manner, the present invention provides a "standard" that allows different laboratories and operators to treat cells in a uniform, repeatable manner so as to maintain the viability of the cells.

If desired, systems and methods according to the invention provide for further standardizing a protocol, particularly a stem cell treatment protocol, while further minimizing the potential for contamination, especially bacterial contamination, of the treated material. For example, in accordance with another embodiment of the invention, the treatment solution, e.g., a cryoprotectant mixture, is supplied as a standardized preformulated sterile solution. Preferably, the solution is preformulated with respect to component concentration and total volume. Since the solution can be supplied as a standardized sterile preformulated solution, this avoids both the variability of individually prepared solutions and the labor intensive effort of such operations. Additionally, the solution can be prepared in advance and stored until needed. Moreover, since the solution is prepared in a closed system, this avoids the potential for contamination caused by open, compromised systems.

Accordingly, the treatment solution container 211 preferably comprises a sterile sealed container having a preformulated treatment solution sealed therein. For example, the treatment solution, e.g., a cryoprotectant mixture, can be prepared in a closed system, and sealed in the container while maintaining sterility. Alternatively, the container can be filled, sealed, and subsequently sterilized.

A variety of treatment solutions can be used in accordance with the invention. In those embodiments wherein the treatment solution comprises a cryoprotectant, the cryoprotectant solution or mixture includes a cryoprotectant agent and mixtures

thereof, such as, but not limited to dimethyl sulfoxide (DMSO), glycerol, polyvinylpyrrolidone, polyethylene glycol, albumin, dextran, sucrose, ethylene glycol, I-erythritol, D-ribitol, D-mannitol, D-sorbitol, I-inositol, D-lactose, choline chloride, amino acids, methanol, acetamide, glycerol monoacetate, and inorganic salts. Exemplary treatment solutions include those disclosed in International Publication No. WO 96/17514.

In typical embodiments, DMSO is used, which is nontoxic to cells in low concentration. It is believed that, being a small molecule, DMSO freely permeates the cell and protects intracellular organelles by combining with water to modify its freezability and prevent damage from ice formation. The addition of plasma (e.g., to a concentration of about 20-25%) can augment the protective effect of DMSO. After the addition of DMSO, cells should be kept at 0°C until freezing, since DMSO concentrations of about 1% are toxic at temperatures above about 4°C.

A variety of suitable treatment solution containers are known in the art. The containers (as well as the seals, stoppers, caps and/or covers) can be constructed of any material(s) compatible with the treatment solution under conditions of use, e.g., processing, sterilization, and/or storage. For example, in those embodiments wherein the treatment solution includes a cryoprotectant such as dimethyl sulfoxide (DMSO), the container is substantially chemically inert to DMSO for the rated storage period, e.g., up to 3 years. In some embodiments, for example, involving the preparation of stem cells, leaching (e.g., caused by the DMSO reacting with the treatment solution container) should be minimized or prevented, so that the cells are as free of extraneous (impure and possibly toxic) material as possible. Extraneous material is undesirable as, for example, it can compromise the viability of the stem cells (initially, during subsequent handling, and/or during transfusion) and adversely impact the recipient of the cells.

Suitable materials for use as containers for storing solutions comprising DMSO include polytetrafluorethylene (PTFE), glass, and ceramics. In one preferred embodiment wherein the treatment solution includes DMSO, the container comprises borosilicate glass.

In those embodiments wherein the treatment solution container has a stopper, seal, cover, and/or cap, at least the portion of the seal, cover, and/or cap contacting the solution is substantially chemically inert to the solution. For example, in an embodiment wherein the treatment solution includes DMSO, and the container includes a rubber stopper, the stopper is lined or faced with PTFE.

While the treatment solution container and the associated cap or cover contacting the treatment solution should be substantially inert to the treatment solution (since the solution is typically stored in the container), other components of the processing set 200, e.g., receiving container 232 and conduit 203, are typically exposed to the treatment solution for lesser periods of time. Additionally, in the receiving container 232, the treatment solution is diluted. Accordingly, these other components should be resistant to the treatment solution, but can be less resistant to the solution than the treatment solution container. For example, in one embodiment wherein the treatment solution comprises a mixture including DMSO, the receiving container 232 and conduit 203 comprise DMSO resistant material such as polyvinyl chloride (PVC) plasticized with a substantially non-blood extractable plasticizer, e.g., trioctyltrimellitate or triethylehexyltrimellilate (TOTM). Additionally, suitable containers and conduits include those produced in accordance with U.S. Patent No. 4,280,497. Typically, the various containers and conduits in the processing set 200 (other than the treatment solution container) are made from plasticized PVC.

In some embodiments, the treatment solution container and/or cover is designed to be punctured, e.g., by a penetrating connector.

If desired, the treatment solution can be transferred from the treatment solution container 211 through the metering system 250 to the receiving container 232 while maintaining a closed sterile system. For example, a bacterial filter that is resistant to the treatment solution can be interposed in the treatment solution fluid flow path between the connector 215 and the receiving container 232. Alternatively, or additionally, container 211 (preferably containing a predetermined volume of treatment solution) can be pre-connected to the metering system 250 before use, or can be connected via sterile docking.

After the treatment solution (e.g., a cryoprotectant mixture) is passed through the metering system 250 into the receiving container 232 to provide a mixture or suspension of white blood cells (including stem cells) and treatment solution, the cell-containing mixture is further processed. Accordingly, and using Figure 3 for reference, the white blood cells suspended in treatment solution are typically passed through conduit 204 into freezing bag 234, and bag 234 is subsequently detached and frozen. Alternatively, in some embodiments, receiving container 232 can be detached and frozen, without using a separate freezing bag.

A variety of suitable freezing bags are suitable for carrying out the invention, and are known in the art. Suitable freezing bags include those disclosed in, for example, International Publication WO 96/17514. In a preferred embodiment, the freezing bag includes a plurality of compartments, typically delimited by heat seal.

The embodiment of the freezing bag illustrated in Figure 3 includes a major portion 234a and a minor portion 234b, wherein each portion communicates with its own access port 228, 229, respectively. Each portion should be provided with indicia thereon (not shown) for identification of the specific unit. If desired, the freezing bag can include a line of demarcation aligned to seal off portions of the bag and defining a scoreline allowing one portion to be severed, without thawing, from the other portion. For example, the cells in the minor portion can be allocated for one use, e.g., culturing, and the cells in the major portion can be allocated for another use, e.g., transplantation.

The freezing bag containing the white blood cells/treatment solution is gradually frozen to an extremely low temperature (e.g., in liquid nitrogen) as is known in the art, for example, as disclosed in U.S. Patent Nos. 5,004,681 and 5,192,553, European Patent EP 0,343,217 B1, International Publication WO 96/17514, and A. Hubel, Transfusion Medicine Reviews, Vol. 11, 1997, pp. 224-233.

Before use (e.g., in analysis, culturing, cloning techniques, and/or transplantation), the frozen cells must be thawed. Typically, the cells are washed to remove or minimize the presence of the treatment solution.

The cells are thawed as is known in the art, and the freezing bag 234 from

processing set 200 (Figure 3) is placed in fluid communication with the post thaw set 300A (Figures 1 and 5) or the post thaw set 300B (Figures 6 and 7).

POST THAW SET

Two embodiments of the post thaw set, illustrated respectively in Figures 5 (post thaw set 300A) and 7 (post thaw set 300B), are described below. As will be described in more detail, the post thaw set is utilized to wash the thawed cells, e.g., to remove or minimize the presence of the treatment solution.

Typically, the thawed cells are passed into the container 344A (Figure 5) or 344B (Figure 7), and wash solution, e.g., from the container 345 (Figures 5 and 7) and/or introduced via at least one port or connector such as connector 342 (Figure 5) or port 386 (Figure 7), is utilized to rinse remaining cells from bag 234 into container 344A or 344B. Additional wash solution can be introduced and utilized to "wash" the cells to remove or minimize the presence of treatment solution. Subsequently, container 344A or 344B is centrifuged to provide a sediment fraction comprising the thawed white cells (including the stem cells), and a supernatant fraction comprising wash solution and treatment solution. As will be described in more detail below, the supernatant fraction is typically passed from the container 344A or 344B into container 345, leaving the white cells in container 344A or 344B for further processing.

The embodiment of the post thaw set 300A illustrated in Figure 5 includes a plurality of conduits 301-308, and containers 344A, 345. The illustrated set 300A also includes two or more connectors 342, 343 (such as spike connectors and/or luer lock connectors), access ports 334-337, and a plurality of flow control devices 31.

Similar to the embodiment shown in Figure 5, the embodiment of the post thaw set 300B shown in Figure 7 includes a plurality of conduits 351-362, as well as containers 344B and 345. The illustrated embodiment of the post thaw set 300B also includes at least one connector 343 (e.g., a spike connector and/or a luer lock connector), access ports 337 and 384-386, and a plurality of flow control devices 31.

For convenience and clarity, the container 344A (Figure 5) and the container 344B (Figure 7) will be referred to below as the "transplant container," and container

345 (Figures 5 and 7) will be referred to below as the "wash container." If desired, the transplant container (344A, 344B) and the wash container (345) can be similar, or even identical in configuration and/or composition. For example, the transplant container 344A and the wash container 345 shown in Figure 5 are similar in configuration. In the embodiment illustrated in Figure 7, the transplant continaer 344B and the wash container 345 have configurations that are more different. Illustratively, in contrast with wash container 345, the transplant container 344B has a length longer than the width, and includes a tapered or funnel-shaped end portion.

The post thaw set can include additional components such as, but not limited to, at least one of a treatment solution metering system, e.g., to transfer wash solution into transplant container 344A, 344B. Other components include, but are not limited to, at least one of a vent, a container, a conduit, a transfer leg closure, a sampling arrangement, and a filter such as a bacterial filter.

Typically, the various containers and conduits in the post thaw set are flexible and are manufactured from plasticized polyvinyl chloride, although other materials (having other characteristics) are suitable for carrying out the invention.

The embodiments of the post thaw set 300A and 300B are typically utilized as follows.

The cells in the freezing bag 234 (Figure 3) are thawed, and the bag 234 is placed in fluid communication with post thaw set 300A (Figure 5) or 300B (Figure 7). The system overviews illustrated in Figures 1 and 6 show freezing bag 234 in fluid communication with post thaw sets 300A and 300B respectively.

For example, and referring to Figures 3, 5, and 7, the connectors 343 (shown with caps) can provide fluid access with major and minor portions (234a, 234b) of freezing bag 234 via access ports 228 and 229. If desired, e.g., in some embodiments wherein the connectors are penetrating connectors, the connectors can include one or more structures such as at least one shoulder or "stop" to more efficiently monitor and/or control the depth of penetration of the connector into the bag 234.

A wash fluid can be provided in wash container 345 (Figures 5 and 7). Alternatively or additionally, wash fluid can be introduced into the post thaw set via

one or more access ports or connectors, e.g., port 337 (Figures 5 and 7), ports 334, 335, 336 (Figure 5), ports 384, 385, 386 (Figure 7) and/or through connector 342 (Figure 5).

The use of wash fluid can improve the efficiency of cell recovery from freezing bag 234 (Figure 3), as the fluid rinses or washes the cells from the bag 234. Typically, the wash fluid is utilized to exchange the treatment solution inside and outside the white cells. For example, the wash fluid dilutes or reduces the concentration of treatment solution (especially DMSO) in the extracellular environment, i.e., the environment surrounding the thawed white cells in a container. The wash fluid can also dilute the concentration of the DMSO within the cells by exchanging with the fluid in the cells. Moreover, as will be described in more detail below, it is sometimes desirable to control the rate and/or volume of wash fluid added to the stem cell mixture so as to promote a gradual change of extracellular fluid concentration and minimize compromising cell integrity by osmotic forces.

A variety of wash fluids are suitable for carrying out the invention. In one embodiment wherein the thawed cells to be washed were previously frozen in a cryoprotectant mixture including DMSO, the wash fluid comprises an isotonic fluid, preferably a colloid, e.g., albumin and dextran in a saline solution. Suitable wash fluids include those disclosed in, for example, U.S. Patent No. 5,192,553, European Patent 0,343,217 B1, and International Publication WO 96/17514.

Typically, the thawed cells are passed from freezing bag 234 into transplant container 344A (Figures 1 and 5) or transplant container 344B (Figures 6 and 7), and some of the cells remaining in the freezing bag 234 are rinsed from the surface of the bag 234 into 344A or 344B using wash fluid. The wash fluid can be repeatedly passed in and out of the freezing bag 234, e.g., to further improve the efficiency of cell recovery.

If desired, additional volumes of wash fluid can be introduced into the post thaw set 300A, 300B. For example, additional "fresh" wash fluid can be introduced to rinse additional cells from the freezing bag 234 into the transplant container 344A, 344B. Alternatively, or additionally, fresh wash fluid can be introduced into the

transplant container to wash the cells, e.g., by decreasing the DMSO concentration inside and outside of the white cells. Illustratively, additional aliquots of wash fluid can be introduced through one or more access ports or connectors as described above. If desired, various aliquots can be introduced through different ports and/or connectors.

For example, using the embodiment illustrated in Figure 5 for reference, an aliquot of wash fluid can be introduced via connector 342 and passed into freezing bag 234 to rinse cells from the bag into transplant container 344A. A subsequent aliquot (e.g., for washing the cells and decreasing the intracellular and extracellular DMSO concentration) can be introduced into container 344A via another port, e.g., port 335.

In some embodiments, at least an initial volume of wash fluid is passed into transplant container 344A or 344B prior to the stem cells being passed from the freezing bag into the transplant container. Alternatively, or additionally, wash fluid can be introduced into the transplant container already having thawed cells disposed therein.

In some embodiment wherein the transplant container 344A or 344B has thawed cells disposed therein, it may be desirable to introduce wash fluid into the transplant container in a controlled manner, e.g., the wash fluid can be transferred into the transplant container at a controlled rate.

For example, using Figure 5 for reference, in an embodiment wherein flow control device 31 associated with conduit 307 comprises an adjustable flow control device (e.g., a screw clamp), the flow control device 31 can be adjusted to provide a desired flow rate. Alternatively, or additionally, conduit 307 can have a selected inner diameter (e.g., to provide resistance) and/or the wash container 345 can be disposed at a selected head height relative to the transplant container 344A during use to provide a desired predetermined controlled flow rate without manually clamping (e.g., constricting the inner diameter of) the conduit to control the rate.

Since the stem cells are now mixed with the wash solution, the flow rate for passing the mixture to the other containers is typically not predetermined. Accordingly, the mixture can be passed from container 344A, to freezing bag 234, and

to wash container 345 using any conventional head height and/or tubing flow resistance.

In accordance with another embodiment, e.g., using Figure 7 for reference, after the wash solution is passed into the wash container 345 (e.g., after passing through conduit 362), the wash solution is passed from the wash container 345 into the stem cell-containing transplant container 344B at a controlled rate. For example, the conduit 359 can have a selected inner diameter, and the wash container 345 can be disposed at a selected head height relative to the transplant container 344B during use to provide a desired predetermined controlled flow rate without manually clamping a conduit. Illustratively, while conduit 359 can have a selected inner diameter in the range of about .03 inches to about .06 inches, conduits 358 and 360 can have inner diameters of, for example, about .15 inches.

Alternatively, or additionally (using the embodiments illustrated in Figures 5 and 7 for reference), wash fluid can be passed from wash container 345 into freezing bag 234 to rinse out remaining cells and the wash fluid and cells can be passed into transplant container 344A or 344B. Once the stem cells are mixed with the wash solution, the flow rate for passing the mixture to another location (e.g., another container) is typically not predetermined. Accordingly, the mixture can be passed from container 344B, to freezing bag 234, and to wash container 345 (e.g., though conduits 353, 358, 360, and 361) using any desirable head height and conventional tubing inner diameter.

In accordance with any of these embodiments, after the cells have been sufficiently washed (e.g., to minimize the intracellular and extracellular DMSO concentration), and sufficiently rinsed from the freezing bag 234 into the transplant container 344A (post thaw set 300A, Figure 5), or 344B (post thaw set 300B, Figure 7), the freezing bag 234 is typically disconnected from the post thaw set, e.g., by sealing and cutting conduit 303 (post thaw set 300A) or conduit 353 (post thaw set 300B).

After the cells are washed, they are typically centrifuged in transplant container 344A or 344B to provide a supernatant fraction comprising wash solution and

treatment solution, and a sediment fraction comprising the white cells (including the stem cells). The supernatant fraction is passed from transplant container 344A (e.g., via conduits 307 and 308), or 344B (e.g., via conduits 356, 355, 354, 358, 360 and 361) into wash container 345, leaving the white cells (in a diluted concentration of cryoprotectant solution) in transplant container 344A or 344B.

The white cells in the transplant container 344A or 344B can be further processed before use. For example, the cells can be diluted to further reduce the concentration of treatment solution present and/or to provide a volume suitable for administration to a recipient.

COLLECTION SET

The previous detailed discussion focussed on processing the white blood cell-containing fluid (i.e., containing the stem cells) after it had been passed into the white cell container 232 of processing set 200 (Figures 1 (or 6) and 3).

However, another embodiment of the invention includes the collection set 100 (Figures 1 (or 6) and 2) for receiving the biological fluid (e.g., cord blood) to be processed to provide the white blood cell-containing fluid that is passed into the receiving container 232.

Figure 2 illustrates an embodiment of the collection set 100 in more detail. The illustrated embodiment includes two connectors 126, 127 (such as phlebotomy needles) in fluid communication with first container 123 via conduits 120-123. A first access port 124 such as an injection port, and a second access port 125 such as a connector port, are interposed between the first penetrating connectors 126, 127, and the first container 123. The illustrated embodiment also includes a vent 138, and flow control devices 31. Each of the components of the illustrated collection set embodiment is described in further detail below.

The first container 123, hereinafter referred to as the "collection container" can be any suitable container. In the illustrated embodiment, the collection container 123 is elongated (e.g., the length is longer than the width) and includes a tapered or funnel-shaped end portion. The collection container can also have any suitable volume. In a

preferred embodiment, the collection container has a volume of about 250 ml or less, e.g., about 200 ml or less.

The collection container 123 can be made of any suitable material, and can be substantially inflexible (e.g., comprising a glass bottle) or flexible. For example, the collection container can be a bag made from plasticized polyvinyl chloride (PVC), e.g., PVC plasticized with dioctylphthalate, diethylhexylphthalate, or trioctyltrimellitate. Suitable containers include those disclosed in, for example, U.S. Patent No. 4,892,537. The collection container may also be formed from polymeric materials such as polyolefins, polyurethanes, or polycarbonates.

The connectors 126, 127 are typically penetrating connectors such as phlebotomy needles as are known in the art. Preferably, as shown in the illustrated embodiment, the collection set includes more than one connector, e.g., to allow the set to be fluidly connected to more than one biological fluid source, and/or to more than one access point. Either or both connectors can be used. For example, both connectors can be used at different locations (e.g., the ends of the umbilical cord) to draw or collect more fluid from the source, or the second connector can be used to draw or collect fluid if the first connector fails to allow the continued collection of fluid (e.g., due to clogging).

The collection set includes a plurality of conduits 120-123 providing fluid communication between elements of the set. Preferred conduits are flexible plastic tubing, e.g., manufactured from plasticized PVC.

If desired, the set can include one or more access ports 124, 125 such as injection ports and/or connector ports, arranged anywhere in the set. These access ports, that can be utilized to connect other components to the set, and/or used while adding and/or removing fluid from the set, are known in the art.

In one preferred embodiment the collection set 100 includes a vent 138. The vent 138 may be used to pass gas or air into, or out of, the collection set. For example, it may be desirable to introduce gas through the vent into the collection set to recover collected biological fluid retained or trapped in a component of the collection set 100, e.g., retained in conduits 122 and 123. The retained or trapped fluid can be

"chased" into the collection container 123 by the gas introduced through the vent 138. Alternatively, gas may be passed out of the collection set 100 via the vent 138. Preferably, the vent 138 prevents bacteria exterior to the set from passing through the vent. For example, suitable vents typically includes a hydrophobic filter medium having a pore rating of less than 0.2 micrometers (μm).

In some embodiments, with or without a vent, the collection set 100 also includes a bacterial filter for passing one or more fluids therethrough. For example, it may be desirable to include a bacterial filter to maintain a closed system in those embodiments wherein one or more fluids are introduced into the set, or additional components are connected to the set.

In an illustrative embodiment wherein the biological fluid to be handled is cord blood, the cord blood is collected and processed in collection set 100 as follows. An umbilical cord is clamped to retain cord blood therein, and one or more connectors 126, 127 such as phlebotomy needles are used to puncture the cord blood vessels, and cord blood is drained therefrom into collection container 123. Typically, collection container 123 includes anticoagulant disposed therein, to prevent coagulation of the collected blood.

If desired, gas accumulated in the set can be passed from the set through vent 138 to recover blood retained in a component of the set such as a conduit 122. Alternatively, or additionally, gas displaced by the blood passing through the set 100 (e.g., into collection container 123) can be passed from the set through vent 138.

In an embodiment, a sedimentation agent such as hydroxyethyl starch is added to the collection container 123, e.g., through access port 124. In some embodiment, e.g., wherein a closed system is desired, the sedimentation agent is passed through a bacterial filter interposed in the fluid flow path between the source of the sedimenting agent, and the access port 124.

The collection container 123, including the cord blood, anticoagulant, and the sedimentation agent, is centrifuged as is known in the art (e.g., as disclosed in International Publication No. WO 96/17514), to form a supernatant fraction comprising white blood cells (including the stem cells) and plasma, and a sediment

fraction comprising red blood cells, some plasma, and the sedimentation agent.

After connecting collection set 100 (Figure 2) with processing set 200 (Figures 1 and 3), the supernatant white cell-rich fraction is passed from the collection set 100 into processing set 200. Illustratively, flow control devices such as clamps associated with the appropriate conduits are opened or closed to allow a suitable flow path, and a pressure differential is created, e.g., by operating an expressor having the collection container 123 disposed therein, causing the supernatant white cell-rich fraction to pass into processing set 200 via conduit 201. The white cell-rich fraction is received in white cell container 232, and processed as described earlier.

In accordance with an embodiment of the invention, the sedimentation of the biological fluid in the collection set 100 can be optimized, e.g., to improve the efficiency of centrifugation and subsequent separation of the white cell-rich supernatant fraction from the red cell-rich sediment fraction.

For example, the size of the collection container 123 can be less than that of a conventional blood bag, e.g., to provide a volume of about 200 ml or less, in contrast with a volume of about 500 ml for a conventional blood bag. Since the container has a reduced volume, the container can be filled more completely, thus minimizing the potential for collapsing and/or folding over during centrifugation.

Additionally, as shown in Figures 8A and B, a support or holding device 700 can be provided to further improve the efficiency of sedimentation and centrifugal separation of the components of the biological fluid. For example, the support or holding device 700 can minimize the potential for container collapse during centrifugation and/or while transferring the container to an expressor. An advantage of minimizing collapse during centrifugation and/or transfer is to allow, and maintain, efficient separation of fluid fractions. Additionally, the support or holding device 700 can allow increased exposure of the biological fluid to centrifugal force during centrifugation by spreading fluid along more of the length of the container. Moreover, the device 700 can improve the exposure of the container to the range of centrifugal force, e.g., while the centrifuge bucket containing the container is extended outward.

As shown in Figures 8A and B, one embodiment of the support or holding

device 700 comprises supports 701,702 such as plates that can be placed facing the major sides of the collection container 123. The supports can be held in position by a retainer arrangement 703, such as one or more straps or bands (e.g., containing an adhesive, or hooks and loops such as Velcro® straps; or rubber bands). The collection container 123, interposed between the supports 701, 702 of the device 700, is subsequently placed in a centrifuge cup, and centrifuged. The container and device can be placed directly in the centrifuge bucket, or in a centrifuge bucket insert that is placed in the cup, with the long axis of the container arranged along the radius of centrifugation.

After centrifugation, the container and device can be placed directly in an expressor, if desired.

Since the container is preventing from collapsing, the efficiency of separation is increased by improving the distribution of fluid in the centrifugal field. Accordingly, the red cells are sedimented more efficiently, particularly in those embodiments wherein a sedimentation agent is utilized to accelerate the settling of the red cells. Moreover, since the bag is prevented from collapsing after centrifugation, the device 700 assists in minimizing the disturbance of the separation interface between the supernatant and sediment fractions during transfer to the expressor and during the subsequent supernatant expression. As a result, more of the white blood cells can be passed with the supernatant fraction into processing set 200.

ADDITIONAL SYSTEM COMPONENTS

In accordance with embodiments of the invention, it can be desirable to take one or more samples (e.g., of a biological fluid component) at any stage(s) of the fluid handling protocol. Samples can be obtained by utilizing one or more access ports disposed anywhere in the system 1000, e.g., in sets 100, 200, 300A and/or 300B. Alternatively, or additionally, as shown in Figure 9B and 9C, the system can include one or more sampling arrangements 500 comprising a container 2 including a resilient portion and having closed end 6 and an open end 7, and a cap 3, wherein the arrangement 500 provides for collecting the sample. The embodiments of the

arrangement shown in Figures 9A and 9B also include conduits 11-13, and conduit connector 20 (Figure 9B only).

In an illustrative method of using the sampling arrangement, the container is compressed and decompressed one or more times to allow the container 2 to fill with the fluid for sampling. The container 2 containing the sample therein can be separated from the set, e.g., after sealing the conduits communicating with the container, without compromising sterility.

The container 2 in the sampling arrangement 500, and/or container(s) 123, 232-234, 344A, 344B and/or 345, include indicia thereon for identification of the specific sample and the source.

EXAMPLE

This Example illustrates the use of a preformulated sterile solution, and the controlled addition of the solution in accordance with an embodiment of the invention.

A cryoprotectant mixture is prepared by combining one volume of 100% dimethyl sulfoxide (DMSO), one volume of 10% dextran 40 (40000 MW) in water, in a precision filled system wherein a borosilicate glass ambot is filled and subsequently sealed with a teflon-faced rubber stopper having an aluminum seal. The ambot has an internal volume of 10 ml, and contains 7 ml of the cryoprotectant mixture, and is sealed maintaining the sterility of the contents, i.e., the ambot is terminally sterilized.

A white cell-enriched, plasma-depleted fluid, comprising stem cells, is prepared in a closed system in accordance with the invention. The receiving container having 20 ml of white cell-enriched, plasma volume-reduced fluid disposed therein is a flexible blood bag.

A cryoprotectant mixture metering system is configured as shown generally in Figure 3, and is placed in fluid communication with the ambot as shown generally in Figures 4 A-E.

The receiving container has a 50 cm length of 0.035" ID microbore tubing integrally attached thereto. Both the tubing and the receiving container are fabricated from polyvinyl chloride resin plasticized with triethylhexyltrimellitate (TOTM).

Attached to the tubing is a vented spike. The spike includes a 0.2 micrometer bacteria retentive vent filter, a plastic base and a beveled-tip needle. The spike also includes a vent cap (for the vent filter) that is initially closed.

Using Figures 4 A-D for reference, a positioning jig 17 is slidably engaged with the ambot wherein extensions 17(e) engage with the collar of the ambot. The jig includes an aperture 17(b) of sufficient size to pass the needle therethrough. The needle is passed through the aperture and penetrates the stopper of the ambot, and the tip of the needle extends 1.3 cm into the interior of the container. The jig is slidably engaged with the handle of the spike wherein the ends of spike handle 213 fit within slots 17(h) as side walls 17(c) move toward the spike.

By using such a jig, the tip of the spike can be reproducibly positioned inside the treatment solution container.

The ambot is inverted, and the vent is uncapped to prime the system. The walls of the receiving container are pulled apart to create a vacuum. The cryoprotectant passes from the ambot toward the receiving container to prime the tubing. Once the cryoprotectant reaches the port of the receiving container, the vent is capped and flow stops. The receiving container, that contains the white cell-enriched fluid, is placed between two cold packs (not shown) on a commercially available agitator or shaker 240.

The ambot, as well as the jig and spike, is positioned as shown generally in Figure 4E using a stand (not shown) to provide a head height of 30 cm. The vent is uncapped, and flow resumes. The resistance of the microbore tubing and the head height provide a controlled predetermined flow rate of about 0.3 ml per minute. The flow stops automatically when the level of cryoprotectant in the ambot reaches the bevel of the needle. Thus, the system delivers a controlled predetermined volume of 5 ml.

All of the references cited herein, including publications, patents, and patent applications, are hereby incorporated in their entireties by reference.

While the invention has been described in some detail by way of illustration and

example, it should be understood that the invention is susceptible to various modifications and alternative forms, and is not restricted to the specific embodiments set forth. It should be understood that these specific embodiments are not intended to limit the invention but, on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention.

What is claimed is:

1. A fluid metering system comprising:

a connector suitable for providing fluid communication with a first container, the first container comprising a treatment solution container having a volume of treatment solution disposed therein;

a conduit in fluid communication with the connector,

wherein the system is dimensioned for transferring from the first container into a second container downstream of the conduit a controlled predetermined volume of the treatment solution that is less than the volume of the treatment solution in the first container.

2. A fluid metering system comprising:

a connector suitable for providing fluid communication with a first container, the first container comprising a treatment solution container and having a defined internal volume;

a conduit in fluid communication with the connector,

wherein the system is dimensioned for transferring from the first container into a second container downstream of the conduit a controlled predetermined volume of the treatment solution that is less than the internal volume of the treatment solution container.

3. A fluid metering system comprising:

a connector suitable for providing fluid communication with a first container, the first container comprising a treatment solution container having a volume of treatment solution disposed therein;

a conduit in fluid communication with the connector,

wherein the system is dimensioned for transferring from the first container into a second container downstream of the conduit a controlled predetermined volume of the treatment solution at a controlled predetermined rate, the controlled predetermined volume being less than the volume of the treatment solution in the first container.

4. A fluid metering system comprising:

a connector suitable for providing fluid communication with a first container, the first container comprising a treatment solution container having a volume of treatment solution disposed therein;

a conduit in fluid communication with the connector,

wherein the system is dimensioned for transferring from the first container into a second container downstream of the conduit the treatment solution at a controlled predetermined rate.

5. A fluid metering system comprising:

a conduit suitable for providing fluid communication with a first container, the first container comprising a treatment solution container having a volume of treatment solution disposed therein,

wherein the system is dimensioned for transferring from the first container into a second container downstream of the conduit the treatment solution at a controlled predetermined rate.

6. A fluid metering system comprising:

a conduit suitable for providing fluid communication with a first container, the first container comprising a treatment solution container having a volume of treatment solution disposed therein,

wherein the system is dimensioned for transferring from the first container into a second container downstream of the conduit a controlled predetermined volume of the treatment solution that is less than the volume of the treatment solution in the first container.

7. The system of claim 1 or 2, wherein the system is dimensioned for transferring the controlled predetermined volume of the treatment solution at a controlled predetermined rate.

8. The system of any one of claims 1-5, further comprising a fitting interposed between the connector and the first container.

9. The system of claim 8, wherein the fitting is dimensioned to engage with the connector and the first container.

10. The system of claim 9, wherein the fitting is re-usable.

11. The system of any one of claims 1-8, wherein the connector comprises a penetrating connector.

12. The system of any one of claims 1-7 for transferring a treatment solution including a cryoprotectant.

13. The system of any one of claims 1-8, or 11, wherein the system includes a vent comprising a porous medium.

14. The system of any one of claims 1-8, or 11, further comprising the fluid treatment solution container.

15. A biological fluid treatment system comprising:

a sealed terminally sterilized container;

a biological fluid treatment fluid mixture including a cryoprotectant;

wherein the sealed terminally sterilized container has the fluid mixture disposed therein.

16. The system of claim 15, wherein the sealed container has a predetermined volume of fluid mixture disposed therein.

17. The system of any preceding claim, suitable for use in a closed system.

18. A method for transferring a treatment solution comprising:

passing a controlled predetermined volume of a treatment solution through a fluid metering system.

19. A method for transferring a treatment solution comprising:

passing a treatment solution through a fluid metering system at a controlled predetermined rate.

20. A method for transferring a treatment solution comprising:

passing a controlled predetermined volume of treatment solution through the system at a controlled predetermined rate.

21. The method of any preceding claim, including automatically stopping the flow of treatment solution through the system.

22. The method of any preceding claim, wherein the treatment solution comprises a fluid mixture including a cryoprotectant.

23. The method of any preceding claim, further comprising passing the treatment solution into a container having white blood cells disposed therein.

24. The method of any preceding claim, carried out while maintaining a closed system.

25. A set for processing a white blood cell-containing fluid comprising:

a first container plasticized with triethylhexyltrimellitate

a second container suitable for freezing white blood cells, wherein said second container is in fluid communication with the first container.

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